

REMARKS

Claims 1-44 were pending prior to this Response, with claims 3, 5, 31, 32 and 42-44 being withdrawn as subject to a restriction requirement. By the present communication, paragraph [0130] of the Specification has been amended to delete an active hyperlink. In addition, no claims have been added, claims 2-3, 5-8, 10, 17-19, 21, 29-33, 37-39 and 42-44 have been cancelled without prejudice, and claims 1, 9, 11-14, 16, 20, 24, 25, 28 34-36 and 40 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1, 4, 9, 11-16, 20, 22-28, 34-36 and 40-41 are currently pending in this application.

The Declaration

The Examiner asserts that the declaration filed January 31, 2003 is defective because it incorrectly claims priority to a non provisional application under 35 U.S.C. § 119(e) rather than 35 U.S.C. § 120. To overcome the rejection, Applicants submit herewith an unsigned Declaration that correctly claims priority to the application Serial No. 09/771,357 under 35 U.S.C. § 120. Applicants will submit an executed copy of the revised Declaration as soon as signatures of the co-inventors have been obtained.

The Objection to the Specification

The Office Action contains an objection to the Specification for containing an embedded hyperlink and/or other form of browser-executable code. To overcome the objection, by the present communication Applicants have amended the paragraph beginning at line 19 of page 32 to remove the active hyperlink.

In addition, the Examiner has requested that either the drawings of the description of the drawings be amended to set forth the proper sequence identifiers for each sequence. In response to this objection to the Specification, Applicants submit herewith copies of Figures 1-10 with

mark-ups showing proposed amendments to Figures to recite the sequence identifiers for the sequences shown in the Figures. Applicant will provide formal drawings that include corresponding amendments to Figures 1-10 upon allowance of claims in this application.

In view of the amendment and the marked-up copies of Figures 1-10 showing proposed amendments to add proper sequence identifiers, Applicants respectfully request reconsideration and withdrawal of the objection to the Specification.

The Rejection under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claims 1-2, 4, 6-31 and 34-41 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. Applicants disagree with the Examiner's assertion that the Specification fails to provide sufficient description to enable those of skill in the art to make or use the invention commensurate in scope with the previously presented claims. By the present communication, previously presented claims 2, 5-8, 10, 17-19, 29-31 and 37-39 have been cancelled without prejudice, rendering the rejection moot as to the subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 4, 9, 11-16, 20, 22-28, 34-36, and 40-41.

The Examiner asserts that the claims do not set forth the relationship between the nucleic acids of a subject and "the same" nucleic acids of a control that is not a comparison of nucleic acids between two individuals. To address the Examiner's concern, Applicants have amended claims 1, 16 and 28 to require detection of a state of hypermethylation in the patient's nucleic acid as compared with "the state of methylation of one or more CpG islands in the promoter of RAR β 2 nucleic acids in comparable samples obtained from normal subjects." Thus, the "control" is not limited to breast tissue of a single normal individual, but is required to be representative of nucleic acids obtained from a plurality of normal, i.e., the absence of

hypermethylation in CpG islands in the promoter of RAR β 2 nucleic acids in a broad population of normal subjects.

Further, the Examiner asserts that there is no teaching or guidance in the specification that hypermethylation in an intron or exon of RARB2 would lead to decreased expression of RARB2 or be associated with breast cancer or any cellular proliferative disorder of the breast, thus causing those of skill in the art to allegedly engage in undue experimentation to practice the invention. However, the invention methods for detecting primary breast cancer, as recited amended claims 1, 16 and 28, require detection of a state of hypermethylation of one or more CpG islands in the promoter of RAR β 2 nucleic acids in the subject's sample as an indication that the subject has primary breast cancer. Thus, hypermethylation in an intron or exon of RAR β 2 is excluded by the claim amendments.

The Examiner acknowledges that the Specification is enabling for an embodiment of the invention described as follows: "a method of detecting primary breast tumors in a subject comprising obtaining nucleic acid from a blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow specimen of a subject and determining the state of methylation of CpG islands of the promoter of RARB2 nucleic acids, wherein hypermethylation of CpG islands in the promoter of RARB2 is indicative of breast cancer in the subject" (Office Action, page 3). To reduce the issues and expedite prosecution, Applicants have amended independent claims 1, 16 and 28 to focus the invention on the subject matter that the Examiner has indicated is allowable. However, Applicants specifically reserve the right to pursue other embodiments of the invention in a subsequently filed application.

In view of the amendments and for the reasons discussed above, Applicants submit that the Examiner's concern that those of skill in the art would have to engage in undue experimentation in order to practice the claimed invention has been overcome. Accordingly,

reconsideration and withdrawal of the rejection of claims under 35 U.S.C. § 112, first paragraph, are respectfully requested.

The Rejection under 35 U.S.C. § 102(a)

A. Applicants respectfully traverse the rejection of claims 1, 4, 28-30, 34, and 37 under 35 U.S.C. § 102(a) as allegedly being anticipated by Ferguson et al. (*PNAS* 97:6049-6054, 2000; hereinafter "Ferguson"). By the present communication, previously presented claims 29, 30 and 37 have been cancelled without prejudice, rendering the rejection moot as to the subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 4, 28 and 34. Applicants submit that the invention methods for detecting primary breast cancer in a subject, as defined by amended claims 1, 28, distinguish over the disclosure of Ferguson by requiring:

determining the state of methylation of one or more CpG islands in the promoter of RAR β 2 nucleic acids isolated from a sample comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof of the subject, wherein a state of hypermethylation of one or more CpG islands in the promoter of RAR β 2 nucleic acids as compared with the state of methylation of one or more CpG islands in the promoter of RAR β 2 nucleic acids in comparable samples obtained from normal subjects is indicative of primary breast cancer in the subject.

By contrast, Ferguson is absolutely silent regarding all elements of the invention methods for detecting primary breast cancer as defined by amended claim 1 and 28. The Examiner asserts that Ferguson discloses that hypermethylation of the sigma promoter, for example, a CpG rich region of the 14.3.3 sigma gene promoter, is largely responsible for silencing of the sigma gene and occurs in a majority breast cancers. However, Applicants respectfully submit that Ferguson fails to disclose that hypermethylation of CpG islands in the promoter of the RAR β 2 nucleic acids, as compared with the methylation of comparable nucleic acids in normal samples, is indicative of primary breast cancer.

As Ferguson fails to disclose each and every element of claims 1, 4, 28 and 34, as would be required to establish anticipation under 35 U.S.C. § 102(a), Applicants respectfully request reconsideration and withdrawal of the rejection over Ferguson.

B. Applicants respectfully traverse the rejection of claims 1-2, 4, 6-31 and 34-41 under 35 U.S.C. 102(a) as allegedly being anticipated by Sirchia et al. (*Oncogene* 19:1556-1563; hereinafter "Sirchia"). Applicants enclose with this Response a Declaration under 35 U.S.C. § 131 stating that the co-inventors of the present application, and of the prior application on which it relies, conceived and reduced the invention methods to practice in the United States prior to the date of publication of the Sirchia article. Moreover, the Declaration states that Nicoletta Sacchi, who is named as one of the co-authors of the Sirchia et al. reference, is one of the co-inventors of the present application and contributed to conception of the invention described in the priority application. By contrast, Silvia M. Sirchia, Anne T. Ferguson, Elena Sironi, Smitha Subramanyan and Rosaria Orlandi, who are named as co-authors of the Sirchia article, contributed to the research effort that led to the Sirchia reference, but did not contribute to conception of the invention as described in the present application or as described in the priority application.

Thus, the disclosure of Sirchia that is relied upon in the rejection of claims 1-2, 4, 6-31 and 34-41 of the present application for alleged anticipation was conceived and reduced to practice by the co-inventors of the present application prior to the date of publication of the Sirchia reference. Accordingly, Sirchia is not available as prior art under 35 U.S.C. 102(a) and reconsideration and withdrawal of the rejection are respectfully requested.

C. Applicants respectfully traverse the rejection of claims 1, 4, 28-30, 34, 37 and 38 under 35 U.S.C. § 102(a) as allegedly being anticipated by Esteller et al. (*Cancer Research*, 58:4515-4518; hereinafter "Esteller"). By the present communication, previously presented claims 29, 30, 37 and 38 have been cancelled without prejudice, rendering the rejection moot as to the

subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 4, 28 and 34.

Applicants submit that the invention methods for detecting primary breast cancer in a subject, as defined by amended claims 1, 28, distinguish over the disclosure of Esteller by requiring:

determining the state of methylation of one or more CpG islands in the promoter of RAR β 2 nucleic acids isolated from a sample comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof of the subject, wherein a state of hypermethylation of one or more CpG islands in the promoter of RAR β 2 nucleic acids as compared with the state of methylation of one or more CpG islands in the promoter of RAR β 2 nucleic acids in comparable samples obtained from normal subjects is indicative of primary breast cancer in the subject.

By contrast, Esteller is absolutely silent regarding all elements of the invention methods for detecting primary breast cancer as defined by amended claims 1 and 28. The Examiner asserts that Esteller discloses a method for determining the methylation state of CpG rich region of the GSTP1 gene promoter using methylation specific PCR in samples of breast tumor from a subject and compared to the methylation status of the nucleic acids in normal breast tissue.

However, Applicants respectfully submit that Esteller fails to disclose a method for determining the presence of primary breast cancer in a subject by determining the presence of hypermethylation of CpG islands in the promoter of the RAR β 2 nucleic acids, as compared with the methylation of comparable nucleic acids in normal samples, as is required in the invention methods.

In re Application of:
Sukumar et al.
Application No.: 10/059,579
Filed: January 28, 2002
Page 15

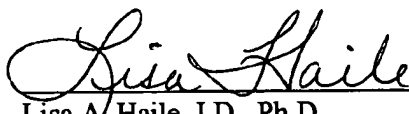
PATENT
Attorney Docket No.: JHU1630-1

Therefore, Esteller fails to disclose each and every element of claims 1, 4, 28 and 34, as would be required to establish anticipation under 35 U.S.C. § 102(a). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection over Esteller.

In view of the above amendments and remarks, Applicants submit that all rejections of the claims are overcome and Applicants request favorable action on all pending claims. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures: Unsigned Declaration of Inventors
Marked-up Copies of Figures (18 sheets)
Declaration of Applicants Under 37 C.F.R. §1.131



(SEQ ID NO:105)

Cyclin D2 promoter, MSP primers
Accn. No. U47284 Promoter region analyzed: -1616 to -1394 bp

1 gagctCGagc caCGcctggc ccGctgcaCG tgccagcttg CGcagcacat cagggCGctg
61 gtctctccc ttctctctgg agtgaatac accaaaggCG GCggtggggg tggggggtga
121 CGggaggaaag gaggtgaaga aCGccacca gatCGtatct cctgtaaaga cagccttgac
181 tcaaggatCG Gttagag ~~gaggtgaaga aCGccacca gatCGtatct cctgtaaaga cagccttgac~~ gCGcctgctt gCGgacttc aCGcagtcCG
241 gctcccaggg agaaagcctg gcagagtgaag gCGGaaacC GgagggtCGG CGaggatgCG
301 ggCGaaggac CGagCGtgga ggcctcatgc ctcCGgggaa aggaagggtt ggtggtgttt
361 gCGcaggggg agCGaggggg agCGGgacct aatccctcac tCGccccctc cccctccCGg
421 gccatttctt agaaagctgc atCGgtgtgg ccaCGgag ~~gaggtgaaga aCGccacca gatCGtatct cctgtaaaga cagccttgac~~ gCGggtCGgct
481 tgtcagcaga tgcagggggCG aggaagCGgg ttttctctgC GtggcCGctg ggCGggggaa
541 cCGctgggag ccttgccccC GgcctgCGgC GgccttagaC GctgcacCGC GtCGccccac
601 ggccccCGaa gagccccag aaacaCGatg gttctgtcC Gaggatcaca ttctatccct
661 ccagagaagc acccccttc ctctctaata cccacctctc cctccctctt ctctctctgc
721 acacactctg cagggggggg cagaagggaC Gttgttctgg tccctttaat CGgggctttc
781 gaaacagctt CGaagttatc aggaacacag acttcaggga catgaccttt atctctgggt
841 atgCGagggt gctattttct aaaatcacc cctcccttat tttcaccta agggacctat
901 ttctaaattg tctgaggtca cccatcttc agataatcta cctacattc ctggatctta
961 aatacaaggg caggaggatt aggatcCGtt ttgaagaagc caaagtggga ggtCGtatt
1021 ttggCGtgct acacctacag aatgagtga attagaggc agaaatagga gtCGgtagtt
1081 ttttgtgggt tgcctgtcCG gggcccttgg catgcaggct ggatggagg agaggggtgg
1141 ggggtggCGg gggacCGCGt ttgaagtggg gtCGggccag ctgctgttct ccttaataac
1201 gagaggggaa agggaggag ggaggagag attgaaagga ggaggggagg acCGggagggg
1261 gaggaaggg gaggaggaac cagagCGggg aggCGCGggg agaggaggga gagtaactg
1321 ccagccagc ttgCGtcacC GcttcagagC GgagaagagC Gaggaggga gagCGagacc
1381 agttttaagg ggaggacCGg tgCGagtgag gcagcccCGa ggtctgtcC gccaccacc
1441 caatcctCGc ctcccttctg ctccaccttc tctctctgcc ctacacctc cccCGaaaaac
1501 cccctattta gccaaaggaa ggaggtcagg gaaCGctct cccctccct tccaaaaaac
1561 aaaaacagaa aaacctttt ccaggcCGgg gaaagcagga gggagagggg cCGcCGggct
1621 ggcc gag

FIGURE 1A



MSP	Unmethylated	223	BP

GT TATGTTATGT TTGTTGTATG

Forward UM 22 BP MT 56

(SEQ ID NO: 21)
(SEQ ID NO: 22)

T AAAATCCACC AACACAATCA

Reverse UM 21 BP MT 56

71 (59)

MSA Member since 1981

THE CHIEF EXECUTIVE OFFICER

F M 19 BP MT 58'

CEG 10 AB:23

CGA-AUTUMN-1996-CPA-MAGE

R M 20 BP MT 56

Case ID NO: 24

MSP External primers · 287 BP

TATT TTGTAAAGA TAGTTTGTAT

Э. IXЭ

TACAACTTCTAAATAACCC

EXT. R

(SEQ ID NO: 129)
(SEQ ID NO: 130)

(SEQ ID NO:130)

FIGURE 1B



(SEQ ID NO:106)

Twist Promoter: Accn No. AC003986

Promoter Region analyzed: nts -51145 TO -51750

1 cattggactg ggtttccttc cacCGaagag tgaactctg cctctttCGa gcaacttcCG
61 aggCGtagtc ctttgatgt tggggagCGt cagactgggt CGttgtagag gggaaaggag
121 gggccagaag ggCGagagag caggCGggga CGcaaatcct cagccccCGC GgCGGcccaC
181 Gtcttcagaa aCGccaggac ctCGgggtg ggcCGcCGG gttggcctt tggaaactcaa
241 gggttCGtct acctgacct tgggtggctc CGCGgttgac actttctttg gcatgcccc
301 ccacccCGCG ccacacacc cccccagcc cagcaatcca aatCGgcccc aCGgacctag
361 agggctcttg ggCGagatga gacatcacc actgtgtaga agctgttgcc attgctgtg
421 tcacagcca tCGgattgg gcttccacCG tggccaggac agtctcttc GacCGcttcc
481 tgggctgCGc taggttCGg gggCGctgc CGcaCGctc GgCGgggaag gaaatCGccc
541 CGGccCGcC GgaggaaggC GaCGgggag gaaggggag ggCGgctagg aggCGgggtg
601 aggggCGgc CGccCGggc agtCGgatt tgaatggtt gggagggaCGa attgttagac
661 ccCGaggaa gagggtggga CGggggagg ggaactgaaa gCGgaaactt tccataaaa
721 cttCGaaaag tccctctcc tcaCGtcagg ccaatgacac tgtgcccc aaactttCG
781 cctgcaCGga ggtataagag cctccaagtc tgcagctctC GcccCGcttc cagacacctc
841 gCGggtctg cagcacCGc accGtttcca ggaggcctg CGgggtgtgC GtccagCGt
901 tggCGcttt ctttttggga cctCGgggc atccacacCG tccccctccc ctccCGctc
961 cctcccCGcc tccccCGCG Gcctctccc CGgaggtccc tccctCGt cctcctgctc
1021 tctctcCGC gggCGcatC GccCGggcCG gCGcCGcC Ggggggaagc tggCGgggtg
1081 aggCGccccG ctcttctct ctgcccCGg ccCGCGaggc caCGGtCGc CGctCGagag
1141 atgcagg aCGtgtccag ctCGccagtc tCGcCGgCG aCGacagcct gagcaacagc
1201 gaggaagagc cagacCGga gacCGcCG agCGgcaagC GCGgggaCG caagCGGCG
1261 aCGagcaggC GcaCGgCGg CGgCGgCGc CGgCGggaCG gagCGgttgg 999CGtCGga
1321 ggCGGCGaCG agcCGggcag cCGgccccag ggcaagCGC gcaagaagtc tGCGggctgt
1381 ggCGGCGgCG gCGGCGCGg CGgCGgCGc Ggcagcagca gCGGCGCGg gagtCGcag
1441 tcttaCGagg agctgcagc GcagCGggtc atggccaaCG tCGGggagCG ccagCGcacc
1501 cagtCGctga aCGaggCGt CGcCGCGctg CGgaagatca tccccCGct gcccCGgac

1561 aagctgagca agattcagac cctcaagctg GCGgccaggt acatCGactt cctctaccag
1621 gtctccaga GCGaCGagct ggactccaag atggcaagct gcagctatgt ggtcaCGag
1681 CGgtcagct aCGcttctC Ggtctgagg atggagggg cctggtccat gtcCGGctcc
1741 cac cagg CGgagcccc caccctctca gcagggCGg agaccCGgt aaggacCGG

FIGURE 2A -- FIGURE 2B



Unmethylated 193 BP
tt TGGatggggt tgggtatTGT FUM (3) 21 BP AT 58 (SEQ ID NO:109)
c ctaaccCAaa CAacCAacc RUM (3) 20 BP AT 60 (SEQ ID NO:110)

Methylated 200 BP
FM (5) 20 BP AT 58 (SEQ ID NO:107)
RM (4) 19 BP AT 58 (SEQ ID NO:108)

External primers 371 BP
Gagatgagatattattttattgtg EXT F (SEQ ID NO:131)
aacaacaatatcatttaacctaac EXT R (SEQ ID NO:132)

FIGURE 2C



RAR beta promoter, MSP primers

ACCN NO. AF157483

(SEQ ID NO: 91)

Promoter region analyzed: nt -196 to nt -357

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1  gtgacagaag tagtaggaag tgagctgttc agaggcagga gggtctattc ttgccaag
61  gggggaccag aattcccat gGagctgtt tgaggactgg gatgcGaga aCGGgCGG
121  gGgGgGgagg gtttgtctgg gcacCGtCGg ggtaggatcC GgaacGcatt CGgaaggctt
181  ttgcaagca tttacttggg aggaagaatt gggatctttc tgggaacccc CCGGgCGGg
241  tggattggcC Gagcaagcct gaaaaatgca attgaaacac agagcaccag ctctgaggaa
301  ctCGtcccaa gccccccatc tccacttctt cccctCGag tgtacaaacc ctgcttCGtc
361  tgccaggaca aatcatcagg gtaccactat ggggtcagCG cctgtgaggg atgtaagggc
421  tttttcCGca gaagtattca gaagaat [REDACTED] atttacactt gtcacCGaga taagaactgt
481  gttattaata agtcaccag gaatCGatgc caatactgtC Gactccagaa gtgctttgaa
541  gtgggaatgt ccaagaatc tgtcaggaat gacaggaaca agaaaaagaa ggagacttCG
601  aagcaagaat gcacagagag ctatgaaatg acagctgagt tggacGatct cacagagaag
661  atcCGaaaaa gtcaccagga aactttccct tcactctgcc agctgggtaa atacaccaCG
721  aattccagtg ctgaccatCG agtcCGactg gacctgggcc tctgggacaa attcagtgaa
781  ctgggccacca agtgcattat taagatCGtg gagtgtgcta aacGtctgcc tggtttcaat
841  ggcttgacca tCGcagacca aattaccctg ctgaaggcCG cctgcctgga catectgatt
901  cttagaattt gcaccaggta taccocagaa caagacacca tgactttctc agaCGgcctt
961  accctaaatC Gaactcagat gcacaatgct ggatttggtc ctctgactga ccttgtgttc
1021 acccttgcca accagctcct gcctttggaa atggatgaca cagaaacagg ccttctcagt
1081 gccatctgct taatctgtgg agacCGccag gaccttgagg aacCGacaaa agtagataag
1141 ctacaagaac cattgtctga agcactaaaa atttatatca gaaaaagaCG acccagcaag
1201 cctcacatgt ttccaaagat cttaatgaaa atcacagatc tCGtagcat cagtgtctaaa
1261 ggtgcagagC Gtgaatttac ctgaaaaatg gaaattcctg gatcaatgcc acctctcatt
1321 caagaaatgc tggagaattc tgaaggacat gaaccttga ccccaagttc aagtgggaac
1381 acagcagagc acagtcctag catctcacc agctcagtgg aaacacagtgg ggtcagtcag
1441 tcaccactCG tgcaataaga ca
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FIGURE 3A



Unmethylated 163 BP
ggattgg gatgtTGaga atGT (SEQ ID NO: 92)
C Aaccaatcca accAaaacAA RUM 21 BP AT 60 (SEQ ID NO: 93)
Retained 162 BP
ggattgg gatgtTGaga atGT FM(2) 19 BP AT 60 (SEQ ID NO: 135)
C Aaccaatcca accAaaacAA RM(2) 19 BP AT 58 (SEQ ID NO: 136)
External primers 266 BP
gtaggagggttttattt tttgtt EXT (2) F (SEQ ID NO: 133)
aattacattttccaaacttactc EXT 4 (2) (SEQ ID NO: 134)

FIGURE 3B



Sequence analyzed: nts +169 to +349
Exon 3 sequence (SEQ ID NO:95)

CCGcagaggC GgCGctgctc ccccaaaCG acaCGGctt ggacCGGg
cagccctggc aggtcCGct ctccaCGgc ctctCGttcc actgCGGgg tgctctggg gaccagagtt ggggtgctgac GgCGGcag

FIGURE 4B

Unmethylated 128 BP

tTgtagaggt Ggtgttgtt Nes1 FUM 20 BP AT 56 (SEQ ID NO:77)
CACACAat aaaaCAaaaa accA Nes1 RUM 22 BP AT 56 (SEQ ID NO:78)

Methylated 161/177 BP

ctccgaat gttctatctcgttttc Nes1 FM 20 BP AT 56 (SEQ ID NO:79)
tctctatctcgttttttccgag Nes1 RM 20 BP AT 58 (SEQ ID NO:80)

FIGURE 4C

UnMethylated 213 BP

tTGgtTGg aagttgggTG FUM 18 BP AT 56 (SEQ ID NO: 71)
gtaTGtg attTGAagTT Gtatt (SEQ ID NO: 98)
aataC AaacttCAaat caCAtac RUM 22 BP AT 56 (SEQ ID NO: 72)

taCGtg attCGaagTC Gtat FM 18 BP AT 58 (SEQ ID NO: 69)
taCGtg attCGaagTC Gtat (SEQ ID NO: 99)
ataAC GaataTCGaataTC CGGtG RM 20 BP AT 56 (SEQ ID NO: 70)

FIGURE 5C



Sequencing 307 BP

Hox A5 Seq. F 23 BP AT 56
 (SEQ ID NO: 73)

ggag ggaattaagt atatgtt (SEQ ID NO:100)

Exhibit A-5
Hox A5 Seq.R 21 BP AT 56
(JEP ID 100:74)

200
 201
 202
 203
 204
 205

Hox Exp F 20 BP AT 60

(SEQ ID NO: 75)

ccaggta cagccagccg gc (SEQ ID NO:101)

Hox Exp R 18 BP AT 62

(SEE 1200:76)

FIGURE 5D



SEQ ID NO: 102 (CONT')

1621 caggattttg ccattctctg cacagcctga gggagactaa caggcctctt tgcagagggg
1681 tagctggtta gaccgtttct tccctgtcgg ccagcactgc ccgtccccct ccacacacca
1741 tctcatcttc atgcgatgcc tgcacaaccc catggagccc gtccatctgt ctggtgtgtg
1801 gtgcggtgtg tgtgctgggt gtggtagggt ctccaggagc tccccgctaa gcagaaggat
1861 cgggatatag gcaaggcta aagccccagc cccattgtgg actgagggaag tacgttcgcg
1921 cagagcagct ctccagctgg aagaggaggt ggaggtgag gctggggaga gcatggcgaa
1981 cctgccctga ggtgcttggg tctgtgctgg tggggtcctg gtatgcaggg gccaccggtc
2041 actaacactc ttatgtcctg gctttctgtc cccgctgagc ttctctcac ccgccggtt
2101 tctctcctgc ttcatgtcct gctgcctaag ccttggccct tctctcggc agaggcagggt
2161 gctgtggcag caccctccc caccaccggg cccctgcagg ccgtctccct cctcccaggc
2221 ctgtctaaccc tctctctctt ccttcttctg tctctgtcgg ggtatctcca gtgtgtcgg
2281 gggcttaagg acctcctgag gaccgtgct ctctgctct ccaggaatgg cctgggggga
2341 gccaggcacc cggcacttcc acctgcctaa cctgtgccc atctgccacc atctgtcct
2401 acagggtctg cccccagcc tggccggcct gtgtgctct taggacccca tagggggcag
2461 gggctggcct ctttgcccca ttcccgctcc atgccggcca ggtgtgtaga agccataacg
2521 cagcagacca tcagcacaat aatgtgactc tacgtgata tctctcctct ctcctccact
2581 gacttcccc tcccggattt gtgaggtgtc aagactagga atctggcctt agagcctgcc
2641 cctccacccc ctacagatcag gcatagccat agtcaagccc agcaggttct ctcaggaggt
2701 gtctgggggtg ttgatggtgg atgacgtgc tgaacaagt ttgtgactgt tctaagcaca
2761 actggcttga tactgttccc acggcctgtc cactctccc ccccaacct ccaccagagt
2821 aggtaggatg tagggagggt gcgtgcgcc ttgtctctag gcactgaggg accaagctag
2881 ccgtgcacag ccccatcac ttcaggggcg taaaggaaag agctgagcca aggaaatca
2941 gctgagccca gggctggggg ctgcttgtct gctatcctgt acctttttt ttttaacca
3001 aaataaagat tcccccttc ttgccatacc attgctgtc tgggtggcgc ttactttgg
3061 ggcccaggga tgggacctgc agtgggctg tggaaacat ggtccccct cgtcccagc
3121 ttcttccag ctggccagtg ctgctctgga gattacaag cacaacgaag ccaggaggga
3181 cacaggaaaa gtggctgaca tcttttccac tctgccccct cagaactctt ggtctcaatt
3241 ccagacacca ccagcctta gctgacctct ggtattctgat aggtccaggt gcagggtgag
3301 acagagggtt taactccagt ttgggactgc catacccatg aactgagccc agcccaggt
3361 aacgatctca tggaaacttc tctctccca gttgctgcac tacatcaaga tacacacatg
3421 tgcatacact gtactatggg ctaaaaaaat acgtaccgt accgttcagc aagggtctgc

FIGURE 6B



(SEQ ID NO: 102 CONT)

3481 cgagtcctcgg gccattttc tcattttaac ctgtgaggag gatgatgtca gcccttttac
3541 agatgaggga actgagactc aaggaagaaa caggagctgc ccaaggctac ccagctggca
3601 aagcagcaaa tcccagatcg gaacctgac tctgccccg gctctgagcc atctgcacta
3661 cccaagggaat gaatacagcg gtgggaggat gagatcttgg agaacccta aaattagaga
3721 atgtcatagc agttagagggg cttagagttg atctgggcca gccctcttgt tttactgatg
3781 gagaaattga agcccagagg caggaaggga cctgcccagg gccattatac agagctggga
3841 tgcagtccta cactctgacc tcattccatt ctctctccat aaattctgca ctgtctctag
3901 actggactgg tttagatgtg ggatactcta aacagcagtg ccttcaagag aaaaagaatc
3961 agaactacga atcactaaa agtaatgtaa gctactctgg gcacactgcc tatggggtcg
4021 cctgtctcca caaggagcca caaaaataat taaaataatt taatatccct tcccaagggt
4081 aaccagtaaa gtaagctctt ggctaggtaa ctggactctt gttcacaaat agccagtggg
4141 aaaagggtgct agagcttctt ctggccacct gtttaatttg atcattccaa gacagaaca
4201 tttcttagga agtctcttct agaattacc tgggtgtccct cccactgcta tcagagccct
4261 gtctctgtc ctcagtggag gttagagaga aatggttgc gcttcttca tcacaacct
4321 tcaaaacctt ttattaccag taaagaagga ttggttgact atgggccaga gccctgagc
4381 ctgctggtag aatggatgct gtacaggagg gtggggagg agcaggcaga atgaggaaaag
4441 cccctttgag ctgcaacccc agctcctgtc ctgctgactc agacagctga ctgtggagct
4501 ccattgccctg ccagggccctg ctgcctctcg cccgtctgag ctctgaact tgggaaatgg
4561 agggccagag gcaaaaggag gtacctgaga caggaactga gtcaggatca acaggccaga
4621 gcgggcagga ggtatcaggc agcctggctc ccagatgcac cctgagctc cagcagggga
4681 ggagtaggaa tgaaggggct tcttgccct tgctcatggc tatgcgagg gcgtgaacca
4741 ccaccaggct ctctggctta agtggcgagg agcaaatggt cctccctgg actcaggctc
4801 caaagtctct gggcctgcct tccaggttcc cagtgtcctg ggatctccag ctttccccag
4861 gacttgggga agccccggct ggatgactag tacaatatga gggccctgag gttccaggac
4921 ctgctgaggt cacaggaata tcctagatca agcttgcca acccacggcc cacaggctgc
4981 atgtggccca gaatggcttt gaatgcagcc caacacaaat tagtaacct tcttaaaaa
5041 ttatgagatt tttttgcaa tttttttt ttttttagct catcagttat tggtagtgtt
5101 ggtatatatt atgtgtggcc caagacaatt ctccaatgt ggcccaggga agccaaaaga
5161 ttggacacgc ctgtcctaga tggagaggga ggaggcagtg ctgagcacat ctggccattc

FIGURE 6C



(SEQ ID NO: 102 cont.)

5221 atccatctgg agagagaagg ctatgggcaa actgtctcct ctccccgtga gacacccagc
5281 tgggaaggct tggcccttgg taagtcctgg ctctgggtcc ttctccattt cacagaaacct
5341 aactctatgt tagtgctttg tgagtatatg ttgatacataa taaagttgac gggatttttt
5401 cacatgataa taatagttgt catctggccg ggcattggtg cttatgccta taatttcagc
5461 actttggaag gctgaggcag gtggatcact tgaggtcagc tgttcgagac cagcctggcc
5521 aacatggtga aaccacatct ctacttaaa aaaaaaaa taaaaaatt agctgggtgt
5581 ggtggtgcac ccttgtaac ccagctactc gggaggctga ggcaggagaa tcacttgaac
5641 ccaggagggt gaggttcag tgagctgaga ttgtgccact acactccagc ctgggtgaca
5701 agagcgaac tccgtctcaa aaaaaagaa aataataata ataatagttg ccatccattc
5761 tactgtgctt tccattaact cgtgtaatcc tcacaagtcc cattttatag ttacaggaac
5821 tgaggctcac agagcttaaa tcacttgcc aaggccaaa agactataa gaattacatt
5881 taggcagtct gattccaaa atactagtct attctgtatc tcatagacaa acaatacata
5941 ttcacttttt tgtgtttgtt ttgttttgag acggagtctt gctctgtcac ccaggctgga
6001 gtgcagtggc gccatctcgg ctcatgcaa cgtccgctc ccgggttcaa gcgattctcc
6061 tgcctcagcc tcccgagtag ctgggactac aggcattgac caccatgccc ggctaatttt
6121 ttgtattttt agtagagaca ggttttctct ggttagcca gaatggtctc gatctcctga
6181 ccttgtgatc caccacctc agcctccaa agtgcctgaga tgacaggcgt gagccaccgc
6241 gtccgacctt tattcactat ttataaattg gagagaataa gaaatcaaa agggccagggt
6301 gtagtgaact acacctgtaa tcccagcact ttgggaagcc aaggcaggag gattgcttga
6361 acccagaagt tcgagaccag cctgggcaac atggtgagac cctgtctcta caaaaaatac
6421 aaaaattagc tgggcgttgt ggtgagcacc ttattcttag gaagctgagg caggaggatc
6481 acctgaggcc aaggagggtt agactgcagt gagctgtgat cataccactg tacttcagcc
6541 tggacatcag agtaagacc tctctaaa aggaaattg agaagaaaga aaatcaaaagg
6601 gaagcaaat cactcactct cactactca agataccctc tagaagtgg tattttagtg
6661 tggttcctat tgttttctgt gtcagtctc tgatttgagc aaaatctttg gfacgtcaaa
6721 cttaaaaacc cctttacttc cttgaaacc ctgtagcatt agccagaca tgtccctact
6781 cctccttgtg gcaaaagaaa ggtatctctc ttgtgtccc agagtcttg ctaagcctc
6841 cctccaggag ggaagatgag tgttcagaca ctgagagttag ctgggggaga cacaggcctg
6901 tgaattatc ctggctcaac tattaggtcg gcagaatccc agtgaaggga gcctacctc
6961 tgagcccat ctaagctttg gctatgggtg ggcagataa gcaggaatcc atccctatag

FIGURE 6D

(SEQ ID NO: 102 cont.)

7021 gctcaatgcc aacacctta ggtgaaactc ttgatgaaac ttgaggccag ggctccggca
 7081 agcaggaaaa gaacgttggc aacagaggtc tccatctctg aggactctgc caggggctcag
 7141 agatggggca atggtcaaaa ggaaggaaca ggccaggcac agtggctcat gcccataatc
 7201 ccagcacctt gggaggctga ggcaggagga tcgcttgagc ccaggagttt gagacctgcc
 7261 tgggcaatgt agtgagatct gctctctatt taaaaaaa aaaaaggaaa gaacaaagtaa
 7321 acttctgaga aacaggctgg gggaggcatc acgtagctgg aattgctgcc ccataaaaca
 7381 gaatggtatg tgtcactgcc acctccctt ctcagtcctc tctctcccca ggttgctagc
 7441 gtccccctgg gggatcaaac tggactgctt cccagcctca gcacagagagc agtctgagtc
 7501 aggcaggaaa gtgggacagc cggggagctg gaccccaccc tctgtgagcc ccgctggtac
 7561 ctgatggcat gtggcttggg gagggcaggt gacctggcgt gaggggccag agggtaaatc
 7621 ctcaaacaa ggtgcaacag ccaccaactt gaaagggaat atgtgtagat gatgggaat
 7681 gtgtccaaac aacctactgg gtgactaatt acaaaggctg ggttgagagc tcagaggctg
 7741 ctgtttaaac acttcattaa gcggcacctc gaaagctgcc acctgcgcac tctgggagct
 7801 cagaggggac cctgaggggg aatgaggcct ggaggatgga accatcttca ggtagactga
 7861 gaaggagcct ggatctcact tccaaacaca gtctggagct cataggtcag aggcctcaat
 7921 gggagaaaa gctaaaggag aggtgtcaga aaggagtctc aggaattgg tggctatgtg
 7981 actttgagca aatctcacc ctctctgaga cttagtgttc ccactctat ggtcctgtgt
 8041 gtgtcacaga gacatggtgg ggattaaatt cgatcgtgat atgaaagtgc ttgggaaact
 8101 ccatggccct acctaaacat gagtatacct cactgaacc aaggggggaa gttacctggc
 8161 aggattagga acccatcct cctgaacctt tatgggctct gtcgaggctg aagcagccag
 8221 gggctaaagc cagtccttag cccctggag ggcactgtga aagtggatct gatttgagaa
 8281 gccgtttcct gatgtgggca gccatgtgat gccagcccc acaagagggg ggcagccctgg
 8341 agcctggaaa ggtgccagt gagggtgggc ccacgcccag atttctctg ctgactgttc
 8401 tgatgattca cccccacac ccagcctttt tacctttact gcagagccgg aaagggtgtg
 8461 ggaagagag gagaggggag caggtcttgg gccctggctc cgccccctgc tctccccac
 8521 ccttctctgg gcctggccac ccagccaaa ggcaggccaa gagcaggaga gacacagagt
 8581 ccggcattgg tcccaggcag cagttagccc gccgcccgc tgtgtgtccc cagagccatg
 8641 gagagagcca gtctgatcca gaaggccaag ctggcagagc aggcggaacg ctatgaggac
 8701 atggcagcct tcatgaaagg cgccgtggag aagggcgagg agctctcctg cgaagagcga

FIGURE 6E



SEQ ID NO: 102 (CONT)

8761 aacctgtctct cagtagccta taagaacgtg gtggcgccg agagggtgctgc ctggaggggtg
8821 ctgtccagta ttgagcagaa aagcaacgag gagggctcgg aggaagaagg gcccaggggtg
8881 cgtgagtacc gggagaagggt ggagactgag ctccaggggc tgtcgacac cgtgctgggc
8941 ctgttgaca gccacctcat caaggagcc ggggacggc agagccgggt ctctacctg
9001 aagatgaagg gtgactacta ccgtacctg gccgaggtgg ccaccgttga cgacaagaag
9061 cgcatacttg actcagcccg gtcagcctac caggaggcca tggacatcag caagaaggag
9121 atgcccggcca ccaaccccat ccgctgggc ctggccctga actttccgt ctccactac
9181 gagatcgcca acagcccga ggaggccatc tctctggcca agaccacttt cgacgaggcc
9241 atggtgatac tgacacacct cagcaggac tctacaaag acgacacct catcatgcag
9301 ctgctgcgag acaacctgac actgtggac gccgacaacg ccggggaaga ggggggcgag
9361 gctcccagg agcccagag ctgagtgtg ccgcccaccg cccgcccctg cccctccag
9421 tccccaccc tgcgagagg actagtatg ggtgggagg ccacccctc tcccctaggc
9481 gctgttcttg ctccaaagg ctccgtggag agggactggc agagctgagg ccacctgggg
9541 ctggggatcc cactcttct gcagctgtg agcgaccta accactgggc atgccccac
9601 cctgtctct cgcaccgct tctcccgc ccaggacca ggctactct cccctcctc
9661 tgcctccctc ctgcccctg tgcctctgat cgtaggaaat gaggagtgc ccgcttgtg
9721 gctgagaact ggacagtggc agggctgga gatgggtgtg tgtgtgtgtg tgtgtgtgtg
9781 tgtgtgcg cgccagtg caagaccgag actgaggaa agcatgtctg ctgggtgtga
9841 ccatgttcc tctcaataa gttcccctg gacctcctc ctgtctctc tccagttctt
9901 ggcgatggc tgggagtgg actggaatc gacttagaga cctgacttt ggacctctga
9961 gttaggggcc tgaactcct aggtggctca gtggcccgca cgcaagactt tgagtcacag
10021 tgaggccggg gtcc

FIGURE 6F



(SEQ ID NO: 103 CONT)

1441 gggtaaggag ttcaaggcag cgccacacc cgggggctct ccgcaaccg accgctgtc
1501 cgctccccc cttcccgccc tccctcccac ctactcattc accaccacac ccaccagag
1561 ccgggacggc agcccaggc cccgggcccc cccgtctcct cgcgcgacac ctggacttcc
1621 tcttgctgca ggaccggct tccacgtgtg tcccggagcc ggcgtctcag cacacgctcc
1681 gctccgggcc tgggtgccta cagcagccag agcagcaggg agtccgggac ccgggcggca
1741 tctgggccc aa gtaggcgc gccgagcca gcgctgaacg tctccaggcc cggaggagcc
1801 gggggggctc cgggtctgag cctcagcaa tgggctccga cgtgcgggac ctgaacgcgc
1861 tgcctccgc cgtccctcc cttgggtggc gggcggtgtg tgccctgct gtgagcggcg
1921 cggcgagtg ggcgcggtg ctggactttg cgcctccggg cgtcteggt tacgggtcgt
1981 tgggcggccc cgcgcgcga cgggtccgc cgcaccccc gccgcggcg cctcactcct
2041 tcatcaaa cagagccgagc tggggcggcg cggagccgca cggaggagcag tgcctgagcg
2101 ccttcaactgt ccactttcc ggccagtcca ctggcacagc cggagcctgt cgtacgggc
2161 ccttcgggtcc tctccgccc agccaggcgt catccggcca ggcagagatg ttctctaacg
2221 cgcctacct gccagctgc ctgagagcc agccgctat tcgcaatcag ggtaagtagg
2281 ccggggagcg cccta

FIGURE 7B



SEQ ID NO: 104 CONT

1681 ccactatcct tgtgggtgga ccaggagtCG gttCGagggt gctccactt agaggtcaCG
1741 CGCGCGtCG ggCGtcttg agacCGtCG gctccctggc tCGtcaCGt gggctcaggc
1801 actactcccc tctacctcc tctCGgtctt taaaagggaag aaggggctta tCGttaagtC
1861 Gcttgtgac ttttcagttt ctccagctgc tggctttttg gacacccact ccccCGccag
1921 gaggcagttg caagCGCGga ggtgCGgaga aataactgcc tcttgaaact tgcagggCGa
1981 ~~gaggcagttg caagCGCGga ggtgCGgaga aataactgcc tcttgaaact tgcagggCGa~~
2041 ctgCGgggca gggctggCGc CGgagcctg agctgcagga ggtgCGctCG ggtcttgggg
2101 caggtggCGg CGgggCGCGC GcCGggagac cccccctaat gCGtctCG ctttctctcaa
2161 ~~gaggcagttg caagCGCGga ggtgCGgaga aataactgcc tcttgaaact tgcagggCGa~~
2221 ctttgagaac attataatga cctttgtgtt atctgcaagc cattatactt gccaCGaat
2281 tctcaagac ~~gaggcagttg caagCGCGga ggtgCGgaga aataactgcc tcttgaaact tgcagggCGa~~
2341 tgcagtcaat ccatcttacc cctggagcaC Ggtccatat acataccttc ctccatgta
2401 gacagccacc atgaatatcc agccatgaca tctatagcc ctgctgtgat gaattacagc
2461 attcccagca atgtcactaa cttggaagggt gggcc

FIGURE 8B

Unmethylated 288 BP

G ggTGtttttg agatTGtTGg FUM 21 BP AT 60 (SEQ ID NO:85)

TG agttgTGaTG ggttttgg (SEQ ID NO:86)

ccaaaacc CATGCAact CA RUM 20 BP AT 58 (SEQ ID NO:87)

FM 18 BP AT 60 (SEQ ID NO:88)

CGggaaaag taCGtgttCG t (SEQ ID NO:89)

RM 20 BP AT 60 (SEQ ID NO:90)

FIGURE 8C





SEQUENCING PRIMERS FOR RASSF1A

External Primers 294 BP

gggagcttgagcttatctgag

RASSF1 ext. F

(SEQ ID NO: 122)

acccctaaacaccccttc

RASSF1 ext. R

(SEQ ID NO: 123)

Internal MSP Methylated 160 BP

gttgatgacgctgagcgcg

RASSF1 FM (2)

(SEQ ID NO: 124)

RASSF1 RM

(SEQ ID NO: 125)

Internal MSP Unmethylated 180 BP

ggttgatttggttgagtg
ctacaacaccttaCtaCaAaCA

RASSF1 FUM
RASSF1 RUM

(SEQ ID NO: 127)
(SEQ ID NO: 128)

FIGURE 10B